AVIAN RESPIRATORY SYSTEM: Overview of Anatomy and Function as Related to Particulate Inhalation

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INTRODUCTION

The avian respiratory system performs the following functions: gas exchange; thermoregulation; phonation; olfaction; air filtration/cleansing; blood filtration; regulation of acid-base balance; and, production and metabolism of blood-borne molecules. This summary will focus first on the macroscopic and microscopic anatomy of the extra- and intra-pulmonary airways and their connections to the air sacs. Patterns of air flow during inspiration and expiration then can be summarized. Finally the defense mechanisms that protect the respiratory system from inhaled particulates and the evidence pertinent to avian particulate inhalation will be reviewed. Extensive reviews of avian respiratory structure and function have been published elsewhere (Jukes, 1971; King and Molony, 1971; Duncker, 1974; Nickel et aI., 1977; McLelland and Molony, 1983; King and McLelland, 1984; Fedde, 1986, 1998; Brackenbury, 1987; Scheid and Piiper, 1987; King, 1993; Brown et al., 1997). Animated images of air flow patterns through the lungs and air sacs can be found at: http://people.eku.edu/ritchisong/birdrespiration.html. The descriptions contained in the present overview pertain primarily to the respiratory system of the domestic fowl.

ANATOMY

Nasal Passages: Depending on the species, the external nasal apertures (**nares**) at the base of the upper beak may be protected by **opercula** (partial or complete flaps) or **cere** and **ricti** (ridges of skin). Feathers arising from the cere may cover the nares. The nasal cavities contain **turbinate bodies** consisting of convoluted mucosa-covered cartilage. The nasal cavities open through the **choana** (medial fissure in the "hard" palate) into the **pharynx** (common passageway for food, water and air). The slit-like **glottis** guards the opening from the pharynx into the **larynx**, and prevents non-aerosol foreign matter (e.g., food and water) from entering the **trachea.**

Conducting Airways: the **trachea** conducts air into the thoracic cavity and bifurcates at the **syrinx** (the avian organ of phonation) to form the right and left **extrapulmonary primary bronchi**. These bronchi penetrate the respective lungs to become the **intrapulmonary primary bronchi** (**Figure 1**). The conducting airways up to this point are reinforced externally with cartilage rings that maintain flexibility while preventing airway collapse. The unilobar **lungs** are located lateral to the vertebral column in the dorsal thorax. The dorsal-lateral border of each lung interdigitates between 5 ribs, thus approximately 25% of the total lung volume is encased between the ribs (**Figures 2 and 3**). Within the lungs of domestic fowl, the **medioventral** (4 each), **mediodorsal** (8 each), **lateroventral** (8 each), and **laterodorsal secondary bronchi** (23-30 each) branch from the intrapulmonary primary bronchus (**Figures 1 and 2**). These secondary bronchi are not supported by external cartilage rings.

Gas Exchange Airways: Arching between the medioventral and mediodorsal secondary bronchi, arcades of long cylindrical **paleopulmonic parabronchi** (tertiary bronchi) (**Figures 2 and 4**) are layered adjacent to one another in a roughly hexagonal array (when viewed in cross section; Stearns et al., 1987). Individual parabronchi are separated from each other by a thin interparabronchial connective tissue septum containing interparabronchial arteries and veins (**Figures 5 and 6**). Approximately 500 paleopulmonic parabronchi are found in each lung of domestic fowl. They measure up to 4 cm long, have a uniform outside diameter of 1.5-2 mm and a lumen diameter of 0.5 mm. Between 100 and 300 freely anastomosing **neopulmonic parabronchi** connect the lateroventral and laterodorsal secondary bronchi (**Figure 4**). Neopulmonic parabronchi measure up to 1 cm long and comprise 20-25% of the total parabronchial volume.

A simple squamous epithelium lines the parabronchial lumen, but this epithelium is not the site of gas exchange. Instead, as shown in **Figures 5 and 6** thousands of **atria** 100-200µm in diameter form pockets projecting 50µm into the luminal wall. The epithelial cells lining the atria produce **surfactant**, which coats the inner surfaces of conducting airways and gas exchange membranes. Spiral bands of innervated smooth muscle underlie the parabronchial luminal epithelium and encircle the opening to each atrium (atrial muscle, **Figure 6**). Elastic fibers encase the walls (septa) and floor of the atria, presumably serving a support function. One or more funnel-shaped **infundibula** penetrate from the atrial floor into the parabronchial wall, with multiple freely anastomosing **air capillaries** originating from each infundibulum (**Figures 5 and 6**). The air capillaries average 8 to 15 µm in diameter and penetrate outward from the infundibulum, extending 200-500 µm to the outer periphery of the parabronchial wall adjacent to the interparabronchial septum (**Figure 6**). Each air capillary is surrounded by a profusion of **blood capillaries** derived from **intraparabronchial arterioles** that branch inward into the parabronchial wall from the **interparabronchial arteries**. Gas exchange occurs at the blood-gas barrier, at the interface between blood capillaries and air capillaries (**Figure 7**).

Air Sacs: Air enters and exits the air sacs via **ostea** that connect with the intrapulmonary primary bronchi, branches of the secondary bronchi, and terminal neopulmonic parabronchi (**Figures 1 and 2**). Domestic fowl possess eight air sacs, including one clavicular, one cervical, two cranial thoracic, two caudal thoracic, and two abdominal sacs (**Figures 1 and 3**). The thin, transparent nonstratified squamous epithelium of the air sacs is poorly vascularized and plays essentially no role in the gas exchange process. The air sac membrane contains small islands of ciliated and secretory cells, and is supported by diffuse elastin fibers (McLelland, 1989). Functionally, the air sacs serve as elastic, inflatable internal reservoirs for "fresh" and "stale" air. In conjunction with the thoracic and abdominal musculature, the air sacs also act in a bellows-like fashion to propel air through the parabronchi. The extensive penetration of air sacs throughout the thorax, abdomen and skeleton accounts for serious concerns regarding carcass contamination that arise when air sacculitis is detected during inspection of poultry at processing plants (King and McLelland, 1984). To simplify further discussion, it is convenient to group the clavicular, cervical and cranial thoracic sacs in the category of **cranial air sacs**, and the caudal thoracic and abdominal sacs in the category of **caudal air sacs**.

AIR FLOW DURING INSPIRATION AND EXPIRATION

Avian lungs remain essentially fixed in volume throughout the *respiratory cycle*, and thus the lungs neither appreciably inflate during inspiration nor deflate during expiration. The current consensus is that all intrapulmonary air channels remain open and relatively fixed in volume throughout the respiratory cycle. Consequently, air must be forced to flow through the intrapulmonary conducting airways by the bellows-like action of the air sacs. A saccopleural membrane is anchored by skeletal muscle (costoseptal muscle) to the internal thoracic wall and covers the ventral lung surface. This membranous structure is penetrated by the ostea to the caudal air sacs and, unlike the mammalian diaphragm, the avian saccopleural membrane does not contribute to the development of a negative intrathoracic pressure. The costoseptal muscles apparently contract during expiration to hold the ostea open (King and McLelland, 1984). Thus birds lack a functional diaphragm and must depend entirely on the contraction and relaxation of thoracic and abdominal muscles during inspiration and expiration.

During inspiration the rib cage and sternum expand to more cranial and ventral positions, increasing the thoracic volume and generating a negative intrathoracic pressure (suction). Simultaneous relaxation of the abdominal muscles coupled with the forward excursion of the sternum and gravitational pull on the visceral organs increases the volume of the abdominal cavity. The resulting negative thoraco-abdominal pressures (-1 cm H_2O) serve to inflate (draw air into) the cranial and caudal air sacs simultaneously (**Figure 8**, upper panel). "Fresh" air enters the trachea and is drawn through the extra- and intra-pulmonary primary bronchi toward the caudal air sacs. This incoming air does not enter the medioventral secondary parabronchi due to their acute caudally-directed angle of insertion along the intrapulmonary primary bronchus. Instead, the incoming fresh air is drawn caudally to: (a) mix with and carry end expiratory stale air from the trachea and primary bronchus, through the neopulmonic parabronchi and into the caudal air sacs; (b) supply the neopulmonic parabronchi and caudal air sacs with fresh air; and, (c) flow through the mediodorsal secondary bronchi, pushing the resident stale air out of the paleopulmonic parabronchi, through the medioventral secondary bronchi and into the cranial air sacs. Thus the caudal air sacs are inflated mainly with fresh air, and the cranial air sacs are inflated mainly with stale air from the paleopulmonic parabronchi (**Figure 8**, upper panel). Throughout the respiratory cycle, ongoing gas exchange occurs between the blood capillaries and air capillaries. Consequently, with the cessation of fresh air inflow at the end of inspiration, parabronchial air once again becomes stale (PCO_2 increases, PO_2 decreases).

During expiration the rib cage and sternum are drawn inward to more caudal and dorsal positions, reducing the thoracic volume and generating a positive intrathoracic pressure. Simultaneous contractions of the abdominal wall muscles reduce the volume of the abdominal cavity. The resulting positive thoraco-abdominal pressures (+1 cm H_2O) *partially* deflate the cranial and caudal air sacs (**Figure 8**, lower panel). The stale air from the cranial air sacs flows through the medioventral secondary bronchi, into the primary bronchus and then cranially out through the trachea. The relatively fresh air in the caudal air sacs is forced cranially, and due to aerodynamic valving most of the air exiting the caudal air sacs first perfuses the neopulmonic parabronchi and then flows through the mediodorsal secondary bronchi. After entering the mediodorsal secondary bronchi, the relatively fresh air flows through the paleopulmonic parabronchi. The stale air that is displaced from the paleopulmonic parabronchi flows, along

with stale air from the cranial air sacs, through the medioventral secondary bronchi into the primary bronchus and out through the trachea (**Figure 8**, lower panel). Aerodynamic valving within the conducting airways insures that the cranial air sacs always serve as a reservoir for *stale* air exiting the parabronchi during inspiration, whereas the caudal air sacs mainly serve as a reservoir for *fresh* air to supply the parabronchi during expiration. This flow of "fresh" air during inspiration and expiration always is unidirectional in the paleopulmonic parabronchi (mediodorsal secondary bronchus to medioventral secondary bronchus), but is bidirectional in the neopulmonic parabronchi (e.g., air flow cessation and reversal occur in the neopulmonic parabronchi during each respiratory cycle, as well as in all air sacs).

As shown in **Figures 6 and 7**, each parabronchus can be modeled as a long tube with air capillaries (resembling the bristles of a bottle brush) radiating outward at right angles from the parabronchial lumen. During inspiration and expiration, rapid convective air flow occurs along the lumen of the parabronchus. Convective air flow may carry air as deep as the infundibula (Stearns et aI., 1987). However, O_2 must move through the gas exchange region of the parabronchus by the relatively slow process of diffusion from the infundibulum to the periphery of the air capillaries, across the **blood-gas barrier**[1], through the plasma, and into the red blood cells (Powell, 1982; Scheid and Piiper, 1987). Blood capillaries carry deoxygenated blood inward (convective blood flow) following the air capillaries back to their junction with the infundibulum near the parabronchus lumen. Because convective air flow occurs longitudinally down the lumen of the parabronchus, whereas blood flow and gas exchange occur in a transverse path across the radius of the parabronchial wall, the pattern of blood flow and air flow in avian lungs has been labeled a cross-current exchange system. When compared with mammalian respiratory systems, the cross-current avian respiratory system permits a higher degree of removal of O_2 from respiratory air, and provides exceptional advantages at low atmospheric pressure (low PO_2), as confirmed by the exceptional tolerance of birds to high altitude. Sparrows are able to fly at an atmospheric pressure of 349 mmHg, corresponding to an altitude of 6100 m, while mice are comatose and nearly unable to crawl under identical conditions (Schmidt-Nielsen, 1975).

RESPIRATORY SYSTEM DEFENSES

Nasal Passages: Feathers covering the nares serve to coarsely filter the incoming air. Turbulent air flow within the nasal passageways forces the inhaled air to swirl over the mucosal surfaces of the turbinate bodies. The air becomes humidified (fully saturated with water vapor), warmed to the bird's body temperature, and cleansed of larger particulates that adhere to the mucus. Additional particulate entrapment is likely to occur as the inhaled air flows through the moist, narrow choanal slit in the hard palate and flows over the moist surfaces of the pharynx and glottis (Hayter and Besch, 1974; Fedde, 1998; Brown et al., 1997).

Conducting Airways: The avian trachea, primary bronchi, and initial roots of secondary bronchi are lined with a **mucociliary epithelium** (a pseudostratified, longitudinally folded ciliated epithelium with mucous-secreting goblet cells). Pathogens and airborne particles become trapped

[1] The blood-gas barrier is composed of the blood capillary endothelium and its basal lamina, the thin air capillary epithelium, and a thin layer of surfactant. In chickens, the endothelium comprises 67% of the barrier thickness, the basal lamina comprises 21%, and the epithelium plus surfactant comprise only 12% of the barrier thickness.

in the mucus, and ciliary action sweeps the mucous cranially (at a rate of 10 mm/min; Fedde, 1998) to the oral cavity where it is swallowed or expectorated (King and Molony, 1971; King and McLelland, 1984). In addition to mucus, the fluids lining avian conducting airways contain antioxidants and surfactant binding proteins that assist in binding and neutralizing inhaled pathogens and antigens (Bottje et al., 1998; Zeng et al., 1998; Johnston et al., 2000). When mammals and birds of similar sizes are compared, the avian trachea is approximately 2.7X longer and has a 1.3X larger radius, which yields a 4X greater tracheal volume. (King and McLelland, 1984). Accordingly, the **mucociliary escalator** has a substantially enhanced opportunity to trap pathogens and particulates in birds when compared with mammals. The mucociliary escalator is an active and highly important line of defense in birds, preventing many aerosol particulates and pathogens from entering the gas exchange parenchyma. For example, poultry reared on floor litter are chronically challenged with air-borne dust, bacteria, and potent antigens (Anderson et al., 1966; Hayter and Besch, 1974; Gross, 1990; Whyte, 1993; Brown et al., 1997; Zucker et al., 2000; Bakutis et al., 2004; Lai et al., 2009). Only modest changes in respiratory function can be detected when broiler chickens (meat-type chickens bred for extremely fast growth and breast muscle accretion) reared on floor litter are compared with broilers reared in much cleaner environments (Bottje et al., 1998; Wang et al., 2002; Lorenzoni and Wideman, 2008). Commercial poultry populations reared on floor litter typically grow rapidly, thrive and reproduce while exhibiting minimal mortality levels. Furthermore, necropsies of clinically healthy broilers reared on floor litter overwhelmingly reveal healthy tracheas, almost pristine air sacs (e.g., uniformly clear and transparent membranes), and macroscopically unremarkable lungs (Wideman et al., 2011).

In commercial poultry the respiratory system becomes dramatically more susceptible to damage if mucociliary transport is inhibited by exposure to noxious gasses (e.g., ammonia) and pathogens such as infectious bronchitis virus (IBV), infectious laryngotracheitis (ILT), avian influenza (AI), Newcastle disease virus (ND), and *Mycoplasma gallisepticum*. For example, IBV causes ciliostasis and distinctive symptoms of upper airway distress (gasping, coughing, gurgling) attributable to obstruction of the trachea by mucus accumulation. Inhibition of the mucociliary escalator in combination with distressed patterns of breathing apparently allow pathogenic bacteria and aerosolized respirable particles to penetrate more readily into the lung parenchyma and air sacs. The ensuing pulmonary inflammation and air sacculitis (infection of the air sacs) are profoundly deleterious (Gross, 1961, 1990; Tottori et al., 1997; Yamaguchi et al., 2000).

Bronchus-associated lymphoid tissues (BALT) constitutively develop in the bronchial mucosa at the junctions of primary and secondary bronchi, and at the ostea to the air sacs of clinically healthy birds (Reese et al., 2006). BALT contain lymphocytes (B cells and T cells), lymphoid nodules, and epithelial cells. The mucosal BALT tissues may functionally compensate for the absence of fully formed lymph nodes in birds, although their specific role remains to be elucidated (Reese et al., 2006).

Gas Exchange Airways and Air Sacs: Whereas the overwhelming majority of airborne particles exceeding 5 µm in diameter are trapped in the nasal cavities and trachea, some of the smaller respirable particles averaging <5 µm in diameter do reach the avian parabronchi and abdominal air sacs (Hayter and Besch, 1974; Mensah and Brain, 1982; Stearns et al., 1987; Fulton et al.,

1990). Respirable particles can be heavily contaminated with a wide range of immunogenic substances including pathogens and toxins (Bakutis et al., 2004). Macrophages and neutrophils play a central role in the mammalian responses to aerosolized particulates, and intra-alveolar macrophages serve as a first line of defense at mammalian gas exchange surfaces. In contrast, healthy birds do not appear to maintain large populations of resident macrophages or other resident leukocytes at their gas exchange surfaces (air capillaries) or within their air sacs, although some macrophages have been detected in the atria and infundibula of the parabronchi, as well as in the larger conducting airways (Maina and Cowley, 1998; Nganpiep and Maina, 2002). The primary phagocytic function within avian parabronchi apparently resides within the epithelial cells lining the atria and infundibula (the same cells that secrete surfactant). These phagocytic endothelial cells engulf particles encountered on their luminal (air space) surface. The internalized particles then may be degraded/digested intracellularly, or they undergo exocytosis to the underlying interstitium. There they are engulfed by resident macrophages located in the spaces between the atrial and infundibular epithelial cells (Stearns et al., 1987; Brown et al., 1997; Reese et al., 2006). Large numbers of macrophages can be induced to enter the air sacs by injecting appropriate antigens or pathogens into the air sac lumen (Fedde, 1998; Reese et al., 2006). During respiratory infection or aspiration of particulates, phagocytic macrophages and heterophils (analogous to mammalian neutrophils) can be found in lavage fluid from the avian respiratory tract, indicating mechanisms do exist that allow substantial populations of phagocytic leukocytes to enter the gas filled spaces when necessary (Ficken et al., 1986; Toth and Siegel, 1986; Toth et al., 1987, 1988; Qureshi et al., 1993; Klika et al., 1996; Lorenzoni et al., 2009; Maina and Cowley, 1998; Nganpiep and Maina, 2002). Intratracheal instillation of *C. parvum or E. coli* effectively increased the number of phagocytes collected by lung lavage within 24 h (Toth et al., 1987). Additionally, macrophages have been reported to migrate into air capillaries in a variety of infectious diseases, including toxoplasmisis, fatal viral hydropericardium syndrome, highly pathogenic infectious bursal disease and highly pathogenic avian influenza (Hower, 1985; Abe et al., 1998; Nakamura et al., 2001). Pathways by which macrophages that have engulfed pathogens or foreign particles are cleared from the lung parenchyma and air sacs remain to be elucidated. Phagocytosed materials may be transported and presented to the local BALT, or they may be transported to peripheral lymphoid organs (e.g., the spleen) (Fedde, 1998; Reese et al., 2006).

Vascular Defenses: Blood-borne particulates and antigens also trigger intrapulmonary immune responses. In addition to particles or pathogens entering the blood stream directly, materials engulfed by lymphatic capillaries subsequently flow through major lymph trunks that empty into the vena cava. Thus the lungs perform the important function of filtering and clearing the returning venous blood of micro- and macro-particulates including bacteria and thrombi, as well as other potent antigens translocated from pathogens resident in the intestine or from sites of infection (Weidner and Lancaster, 1999). In some mammalian species blood-borne antigens are primarily removed from the blood stream by pulmonary intravascular macrophages (PIMs), which are large mature macrophages bound to the pulmonary capillary endothelium. However, resident PIMs are not present in chickens (Lund et al., 1921; Winkler, 1988; Staub, 1994; Warner et al., 1994; Brain et al., 1999; Weidner and Lancaster, 1999). The absence of PIMs does not leave chicken's lungs immunologically unresponsive to blood-borne antigens because the entire blood volume and thus all of the circulating leukocytes flow through the lungs (e.g., the lungs receive 100% of the cardiac output via the pulmonary circulation). For example,

intravenously injected cellulose microparticles (30µm diameter) become entrapped in inter- and intra-parabronchial pulmonary arterioles of broiler lungs. Within 20 minutes post-injection the microparticles trigger marked pulmonary inflammatory responses, including perivascular infiltration of mononuclear cells in combination with luminal accumulations of macrophages. During the ensuing 48 hours occlusive particles are surrounded by granulomatous tissue consisting primarily of macrophages, giant cells, and fibrous tissue. Subsequently virtually all of the microparticles are cleared from the lungs within approximately 3 weeks post-injection, the inflammatory response subsides, and the lung parenchyma again returns to an entirely normal (e.g., non-inflamed, unobstructed) histological appearance (Wideman et al., 2002, 2007, 2011a,b; Wang et al., 2003; Hamal et al., 2008, 2010). Avian lungs possess an impressive ability to eliminate (digest), clear (remove), or segregate (wall off) offending particulates.

DISTRIBUTION, DEPOSITION AND CLEARANCE OF INHALED PARTICULATES: RELEVANT RESEARCH SYNOPSIS

Peacock and Peacock (1965) injected finely ground asbestos fibers suspended in tributyrin (a triglyceride ester of glycerol and butyric acid) into the clavicular air sacs of adult White Leghorn chickens. The injected material spread throughout the air sac and entered the lung parenchyma. Immediate responses were inflammatory, with macrophages engulfing the asbestos fibers and clearing them from the air sacs (presumably into sub-epithelial spaces). Neoplastic and granulomatous tumors formed near the site of injection in 4 out of 30 injected birds. The granulomatous tumor contained asbestos fibers. Evidently the majority of injected birds lived for >3 years. Necropsies conducted 4 years post-injection revealed asbestos fibers remaining in the lung parenchyma, and "asbestos bodies" (asbestos fibers engulfed by macrophages or encased in mineralized connective tissue) were indentified in the "interalveolar septa" (presumably the interatrial septa where clusters of resident macrophages have been demonstrated in chickens by Reese et al., 2006).

Hayter and Besch (1974) evaluated the distribution of aerosolized spherical particles in spontaneously breathing adult roosters. Larger particles ($\geq$3.7µm diameter) primarily were deposited in the nasal passageways and cranial segment of the trachea, although a portion of these particles also entered the caudal air sacs. Smaller particles ($\leq$1.1µm diameter) tended to avoid entrapment in the upper airways and instead were distributed to the lungs and caudal air sacs. Particles were considered to accumulate preferentially at locations where branching of the conducting airways (e.g., rapid amplification of the cumulative luminal cross-sectional area caudal to the syrinx) caused abrupt reductions in air flow velocities, or where reversal of air flow occurred (e.g., in the caudal air sacs) (Hayter and Besch, 1974).

Brambilla et al. (1979) retrospectively evaluated pulmonary lesions in tissues saved during routine necropsies of 11 mammalian and 8 avian species that had chronically inhaled air containing high levels of silicate particles (1 to 10µm in length) while residing at the San Diego Zoo. All of the avian species exhibited severe silicate dust deposition in the tertiary bronchi (parabronchi), accompanied in some individuals by the formation of large granulomas composed of crystal laden macrophages. Fibrosis and necrosis were absent, and none of the birds had been reported to have respiratory problems. Particles deposited in the conducting airways evidently

were effectively cleared by mucociliary escalator, whereas those engulfed by parabronchial epithelial cells or macrophages were much more difficult to clear and, consequently, triggered ongoing immunological responses. When compared with mammals, all of the avian species evaluated in this study appeared to be more susceptible to parenchymal silicate dust retention and granuloma formation (birds were less capable of clearing particulates reaching the non-ciliated secondary and tertiary bronchi), but birds were significantly less susceptible to pulmonary fibrosis (Brambilla et al., 1979).

Mensah and Brain (1982) evaluated the deposition and clearance rates for aerosolized particles (< 0.8µm diameter) in unanesthetized spontaneously breathing hens. Particles of this size were only sparsely deposited in the trachea but considerable deposition was detected in both lungs. More particles accumulated in caudal than cranial portions of the lungs, presumably reflecting preferential particle deposition in the neopulmonic parabronchi where air flow velocities decrease and then abruptly reverse direction. Almost half of the particles had been cleared from the lungs within 1 hour post-inhalation, and 65% of the particles were cleared from the lungs within 12 hours. This rapid phase of clearance presumably reflects the activity of the mucociliary escalator, which appears to be considerably more vigorous in birds than the more sluggish clearance rate for similarly sized particles deposited in mammalian lungs. As particles were cleared from the lungs they accumulated in the gastrointestinal tract (presumably after the tracheal mucus was swallowed) and were eliminated in the feces. Approximately 35% of the particles persisted in the lung parenchyma through the end of the study (36 hours), presumably reflecting the proportion engulfed by parabronchial epithelial cells and interstitial macrophages. Particles also accumulated in pneumatized bones that are penetrated by cranial air sacs, indicating significant numbers of particles streamlined completely through the paleopulmonic parabronchi and thus were dispersed into the cervical and clavicular air sacs (Mensah and Brain, 1982).

Nakaue, Pierson and Helfer (1982) and Bland, Nakue, Goeger and Helfer (1985) evaluated the performance and health responses of broiler chickens exposed to Mount St. Helen's volcanic ash (VA; particles ranging from 0.5 to 10 µm diameter). The VA was applied directly to the wood shavings litter on the pen floor, or was blown daily (for 20 consecutive days) into pens with resident birds. When compared with unexposed control birds, none of the modes of VA exposure altered any of the routine indices of broiler performance, including final body weights, feed conversion, carcass quality, and cumulative mortality. Litter moisture and ammonia levels also were unaffected by VA, suggesting the absence of significant damage to the kidneys and gastrointestinal tract. Aerosol induction of VA did not alter the histological appearance of the turbinate bodies or the trachea, but pathological changes within the lungs were detected in a portion of the birds beginning 4 days post-exposure. Macrophages initially phagocytized the VA dust within secondary and tertiary bronchi. More chronically, a mild lymphoid hyperplasia developed, including the formation of granulomas containing giant cells surrounding phagocytized crystalline material (Nakaue et al., 1982; Bland et al., 1985).

Stearns et al. (1987) exposed spontaneously breathing adult female ducks to aerosolized iron oxide (0.18µm diameter). The ducks were euthanized 24 hours post-exposure, and transmission electron microscopy was used to evaluate particle deposition within the parabronchial parenchyma. Particle clearance from the parabronchial lumen followed a distinctive sequence:

(a) entrapment in the relatively thick layer of surfactant; (b) phagocytosis by the luminal surface membranes of atrial and infundibular epithelial cells (the same cells that secrete surfactant); (c) movement of the phagosome to the basal-lateral surfaces of the epithelial cells; (d) exocytosis of the particles into the interstitial spaces; and, (e) phagocytosis of the particle by atrial and infundibular interstitial macrophages (macrophages were not seen on the epithelial/luminal surface). The disposition of the particles after their phagocytosis by interstitial macrophages was not assessed. Relatively few particles were observed in the air capillaries *per se*, leading to the interpretation that aerosolized particles were distributed to the atria and infundibula primarily by convective air flow (Stearns et al., 1987).

Brown et al. (1997) reviewed the structure and function of avian respiratory system in relation to its susceptibility to damage by inspired particles and toxins. Deposition patterns for aerosolized particles of different sizes and shapes were predicted based on the anatomy of the airways and the physical forces acting on the particles (e.g., inertial forces, gravitational sedimentation, and Brownian diffusion). Inertial impaction was predicted to clear larger particles primarily in the nasal passageways, pharynx, larynx, trachea, syrinx, and points where secondary bronchi branch from intrapulmonary primary bronchi. Gravitational sedimentation and Brownian diffusion were predicted to occur where air velocities are low and particle residence time is prolonged, particularly within the air sacs and parabronchi (Brown et al., 1997).

SYNTHESIS FROM THE AVAILABLE INFORMATION

1. Particle size distributions for the Libby Amphibole (LA) in duff (**Figure 9**) indicate that, if suitably aerosolized, well over half of these particles are small enough to be distributed throughout the avian respiratory system, including to the level of the parabronchial atria and infundibula.

- Ground foraging birds are likely to stir up the duff and kick LA particles into the air; the worst case scenario is created by dust-bathing birds.
- The LA particles may not be easily aerosolized during foraging or dust bathing, but some of the smallest particles may adhere to other inspirable "dust" that more readily becomes suspended as a colloid in the air when the duff is disturbed.

2. Over a period of months or years some of the LA particles are likely to be inspired by ground dwelling/foraging birds.

- Particles trapped in the protective mucus of the nasal passageways, pharynx and ciliated conducting airways will have little biological impact on those structures, and will be cleared rapidly by the mucociliary escalator. Mucus containing particles cleared from the upper airways will be swallowed, enter the gastrointestinal tract, and excreted in the feces. Evaluation of LA content within the core matrix of avian fecal pellets collected within the zone of contamination may constitute the simplest way to directly quantify the possibility that a threat exists.
- Particles deposited in the parabronchi will be phagocytized predominately by epithelial cells that line the atria and infundibula, but also by resident macrophages in the lumen and interstitial macrophages. Engulfed particulates composed of substances that cannot be degraded or digested intracellularly by the epithelial cells and interstitial macrophages

appear to pose a specific problem for birds: the epithelial cells (and apparently the interstitial macrophages) remain *in situ*, presumably emitting modulators (cytokines and chemokines) that provoke ongoing focal inflammatory reactions. The result in some birds appears to be granuloma and giant cell formation at sites where engulfed particulates cannot be cleared from the secondary and tertiary bronchi.

- The pattern of response to embedded particulates does not include fibrosis in birds; mild focal fibrosis would have little functional impact on the non-inflating avian lung, but fibrosis might modestly increase respiratory effort if the air sacs are affected.
- Particles deposited in air sacs are likely to be engulfed by macrophages and cleared from the air sacs. The fate of the responding macrophages, and thus sites to which they might redistribute the LA particles, is not known.

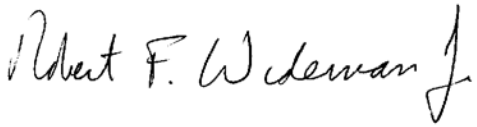
3. There is no evidence that the lungs of wild avian species are anatomically, physiologically or immunologically more susceptible to inhaled particulates than mammalian lungs.

- Published assertions that "avian" lungs are more susceptible to particulate or pathogen damage than mammalian lungs consistently cite examples of the susceptibility of poultry (particularly broiler chickens and modern hybrid turkeys) to respiratory pathogens or to extremely challenging air quality when commercial growout facilities are poorly managed. Indeed, chickens bred for extremely rapid growth and meat production (broiler chickens) provide an excellent model of genetically-imposed cardio-pulmonary and immunological inadequacies. Broiler chicks typically hatch at a weight of 40 g and grow to 4 kg within 8 weeks. Thus in two months a broiler's body weight doubles and redoubles almost 7 times. If human infants grew at the same rate, their body weight would increase from 3 kg (6.6 lb) at birth to 310 kg (690 lb) by 2 months of age. The consequences are obvious: extremely rapid early growth in broilers imposes proportional challenges to their developmentally immature pulmonary, cardiovascular and immunological systems. Rapid growth triggers a suite of "metabolic diseases" attributable primarily to "outgrowing cardio-pulmonary capacities" or "impaired immuno-competency". Wild birds and the progenitors of modern poultry breeds are uniformly found to be considerably more robust than modern broiler chickens and hybrid turkeys (Wideman, 2000, 2001; Nganpiep and Maina, 2002; Wideman et al. 2004, 2007).
- Particulate deposition due to gravitational sedimentation and Brownian diffusion most likely will occur where air velocities are low, particle residence time is prolonged, and at sites of air flow reversal. Accordingly, particles are highly likely to be deposited throughout the alveoli of mammalian lungs, precisely at the level where gas exchange must occur, and where membrane fibrosis is highly detrimental due to the loss of elasticity (alveoli must inflate and deflate during the respiratory cycle; fibrosis significantly increases respiratory effort in birds). In contrast, convective air flow does not penetrate the gas exchange capillaries of avian lungs, thus particle deposition within the air capillaries should be minimal or non-existent. Within the avian lung parenchyma, air flow is bidirectional in neopulmonic parabronchi which comprise 25%, at most, of the lung volume.
- Interstitial inflammation, granuloma development and giant cell formation are normal patterns of avian responses to pulmonary entrapment of particulates delivered either via the inspired air or via the bloodstream. Absent respiratory disease attributable to

pathogens, all available evidence indicates these intrapulmonary inflammatory responses have minimal impact on the function or viability of affected birds.

- Assuming equal levels of "exposure", the above considerations indicate that otherwise healthy mammals are likely to be *more* sensitive to particle inhalation than clinically healthy birds.

4. Conclusion: The experiments conducted by Nakaue, Pierson and Helfer (1982) and Bland, Nakue, Goeger and Helfer (1985) are highly instructive: 20 consecutive days of intensive aerosol exposure to volcanic ash particles of a respirable size did elicit intrapulmonary histological changes but failed to alter any routine indices of broiler performance, nor was mortality affected. Broiler chickens are considerably less robust than wild birds (*vide supra*). Peacock and Peacock (1965) demonstrated that most adult Leghorn chickens survived several years after milligram quantities of asbestos fibers were instilled directly into their air sacs and (presumably) into the lung parenchyma. It is my opinion that some birds in the affected area are likely to exhibit histological evidence of intrapulmonary LA particulate exposure, but that little or no impact on the physiological function or viability of resident avian populations will be discernable.



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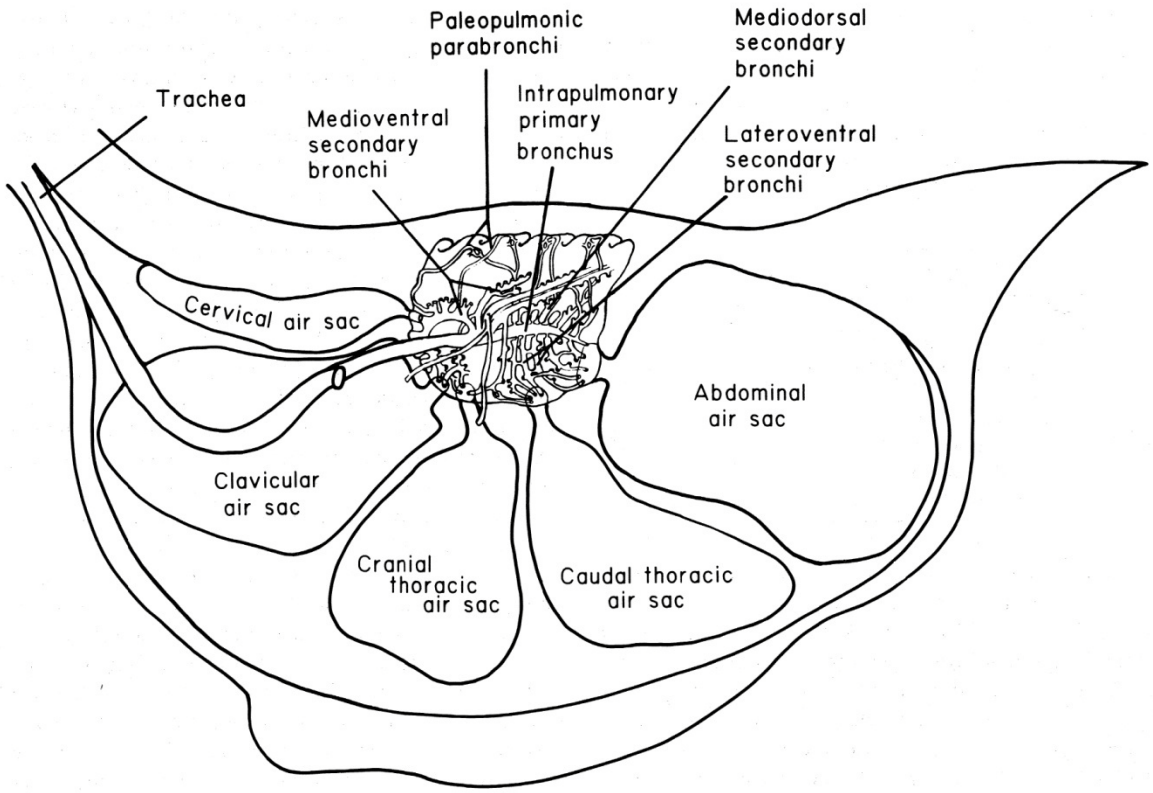
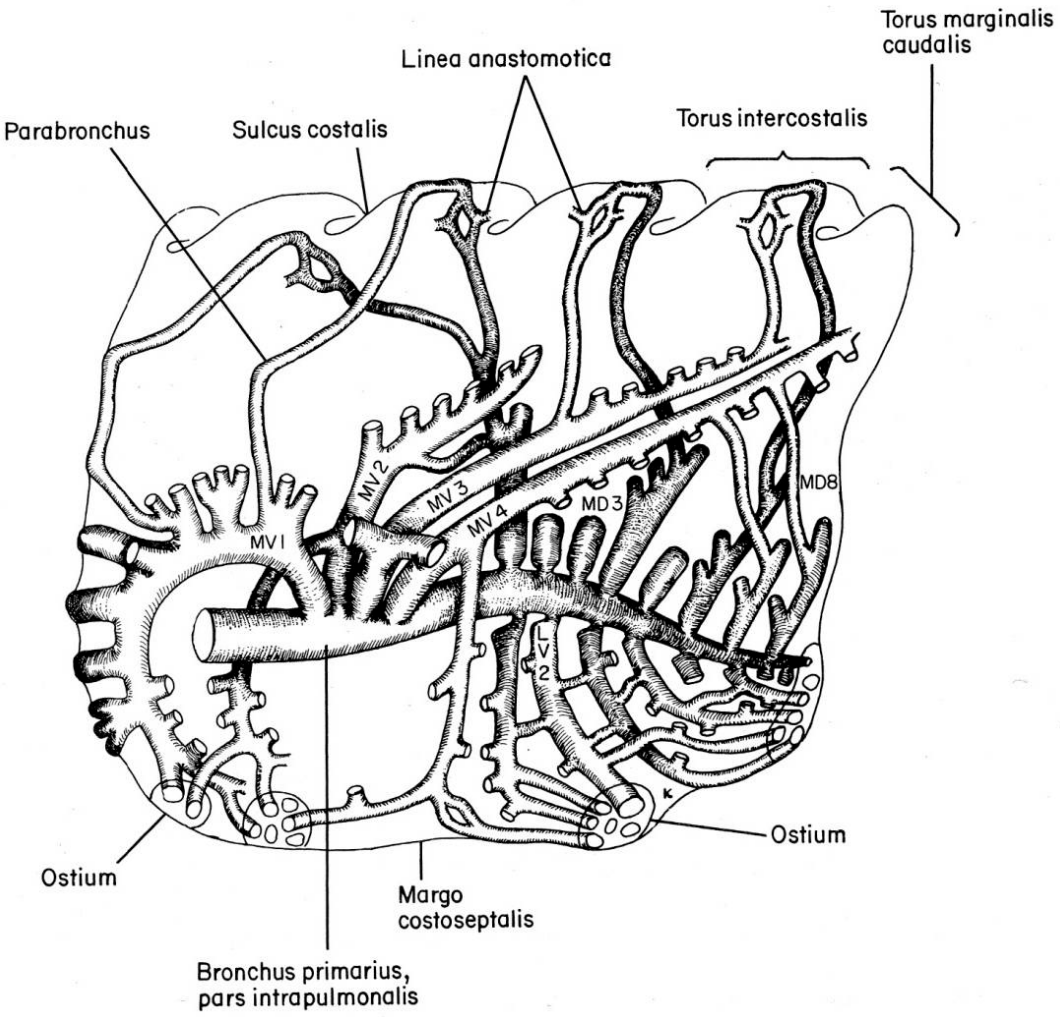
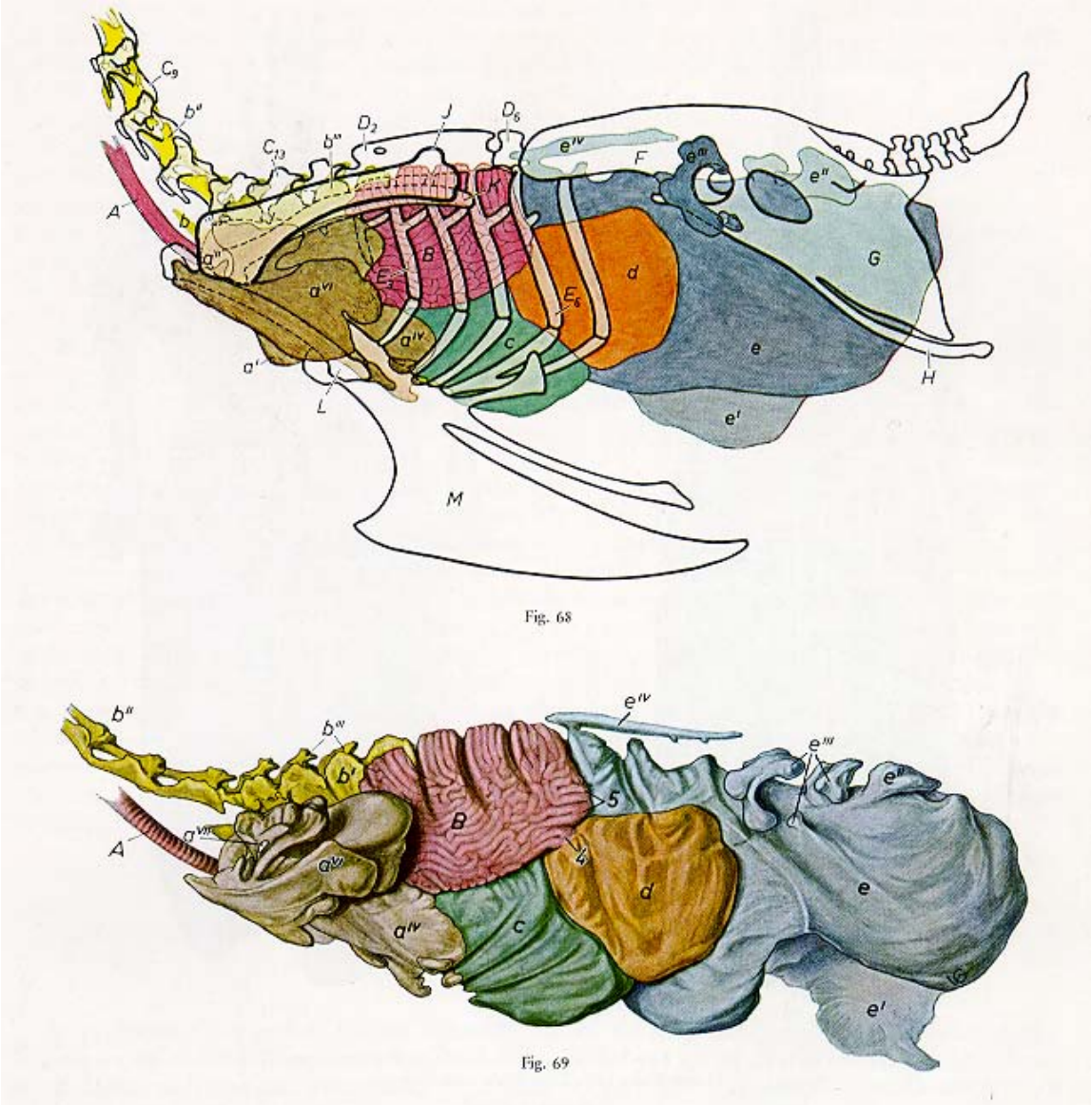


Figure 1. Schematic arrangement of avian lungs and air sacs. Deep within the thoracic cavity the **trachea** bifurcates at the syrinx (the avian organ of phonation) to form the right and left extrapulmonary primary bronchi. These bronchi penetrate the respective lungs to become the **intrapulmonary primary bronchi**. Within the lungs of domestic fowl, the **medioventral**, **mediodorsal**, **lateroventral**, and **laterodorsal secondary bronchi** branch from the intrapulmonary primary bronchus. The bronchi and air sacs connect via ostea.



Medial view of the right lung illustrating: the intrapulmonary primary bronchus; the medioventral (MV), mediodorsal (MD) and lateroventral (LV) secondary bronchi, paleopulmonic parabronchi (tertiary bronchi) connecting the MV and MD secondary bronchi; and, ostea (openings) to air sacs. The Costal sulcus represents a rib indentation.

Figure 2. Details of the primary and secondary bronchi within avian lungs. The **intrapulmonary primary bronchus** penetrates from the cranial to the caudal margins of the lung, opening caudally into the osteum of the abdominal air sac. Within the lungs the **secondary bronchi** branch from the intrapulmonary primary bronchus.



Air sac system of the fowl (Weik, 1963).

A: trachea, B: lung; C: cervical vertebrae; D: thoracic vertebrae; E: ribs; F: ilium; G: ischium, H: pubis; J: humerus; K: scapula; L: coracoid; M: sternum; a: clavicular air sac; b: cervical air sacs; c: cranial thoracic air sac; d: caudal thoracic air sac; e: abdominal air sac

Figure 3. The non-inflating avian lungs (B) are partially encased by 5 ribs (E) as indicated by the costal sulci (indentations) in the dorsal-lateral aspect of the lungs. The air sacs are shown in their anatomically correct positions.

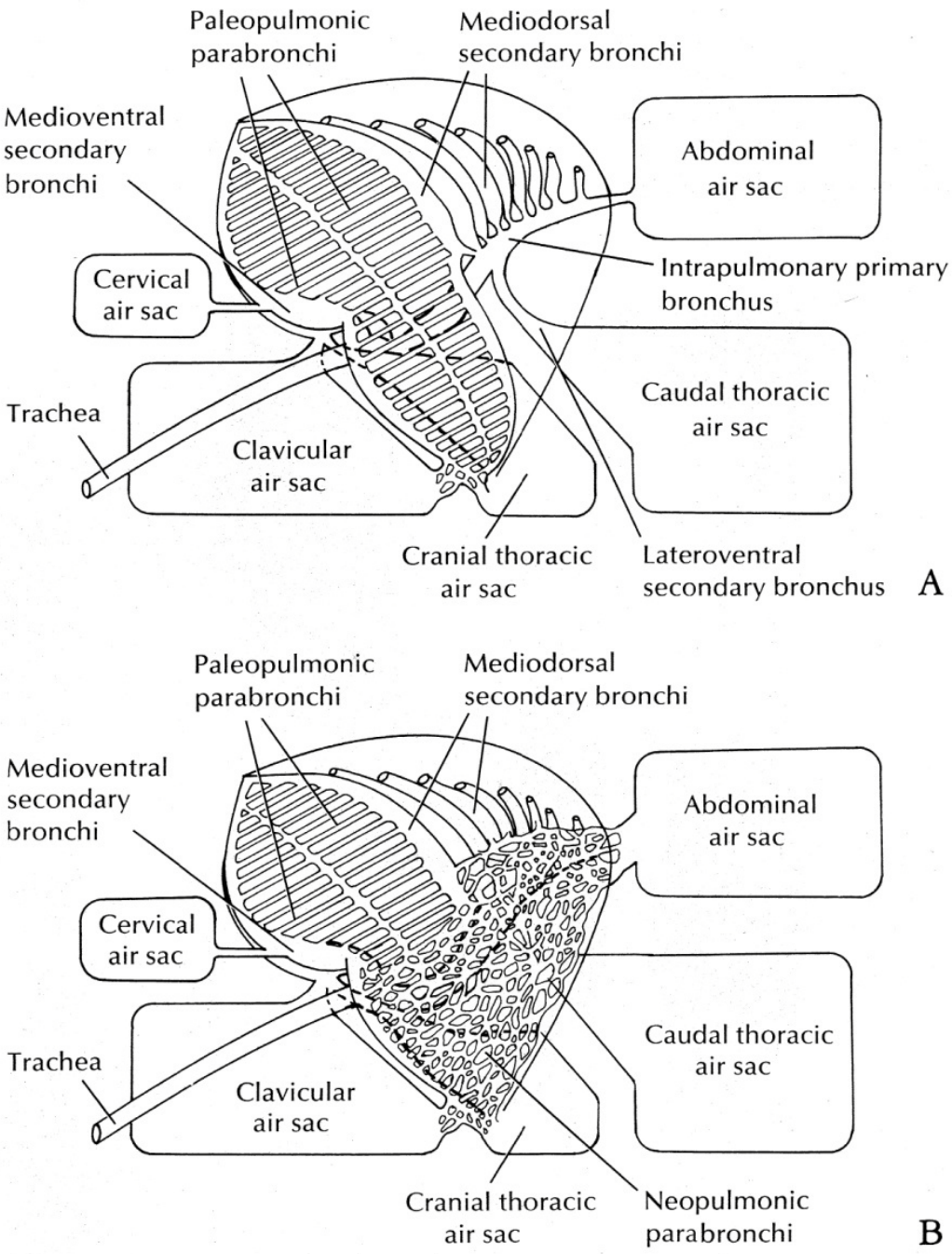


Figure 4. Scheme of the organization of the parabronchi in birds.
(A) Only paleopulmonic parabronchi are present in some birds (e.g., penguin and emu). (B) In addition to paleopulmonic parabronchi, a variably developed net of neopulmonic parabronchi is present in most birds (Duncker, 1972).

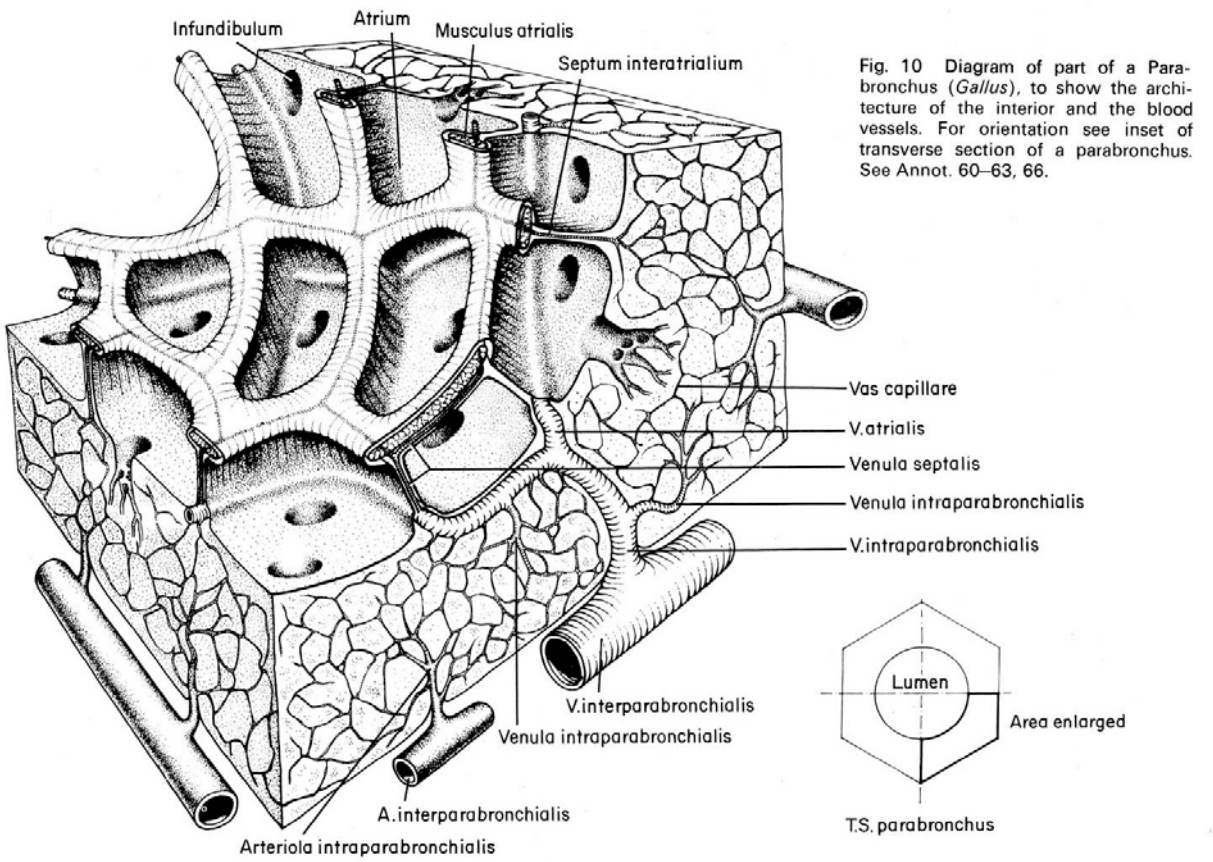


Fig. 10 Diagram of part of a Parabronchus (*Gallus*), to show the architecture of the interior and the blood vessels. For orientation see inset of transverse section of a parabronchus. See Annot. 60–63, 66.

Figure 5. Section through part of the wall of a parabronchus. A**tria** 100-200µm in diameter form pockets projecting 50µm into the luminal wall. Spiral bands of smooth muscle (Musculus atrialis) underlie the parabronchial luminal epithelium and encircle the opening to each atrium. One or more funnel-shaped **infundibula** penetrate from the atrial floor into the parabronchial wall, with multiple freely anastomosing **air capillaries** originating from each infundibulum and radiating outward toward the periphery (outer boundary) of the parabronchus.

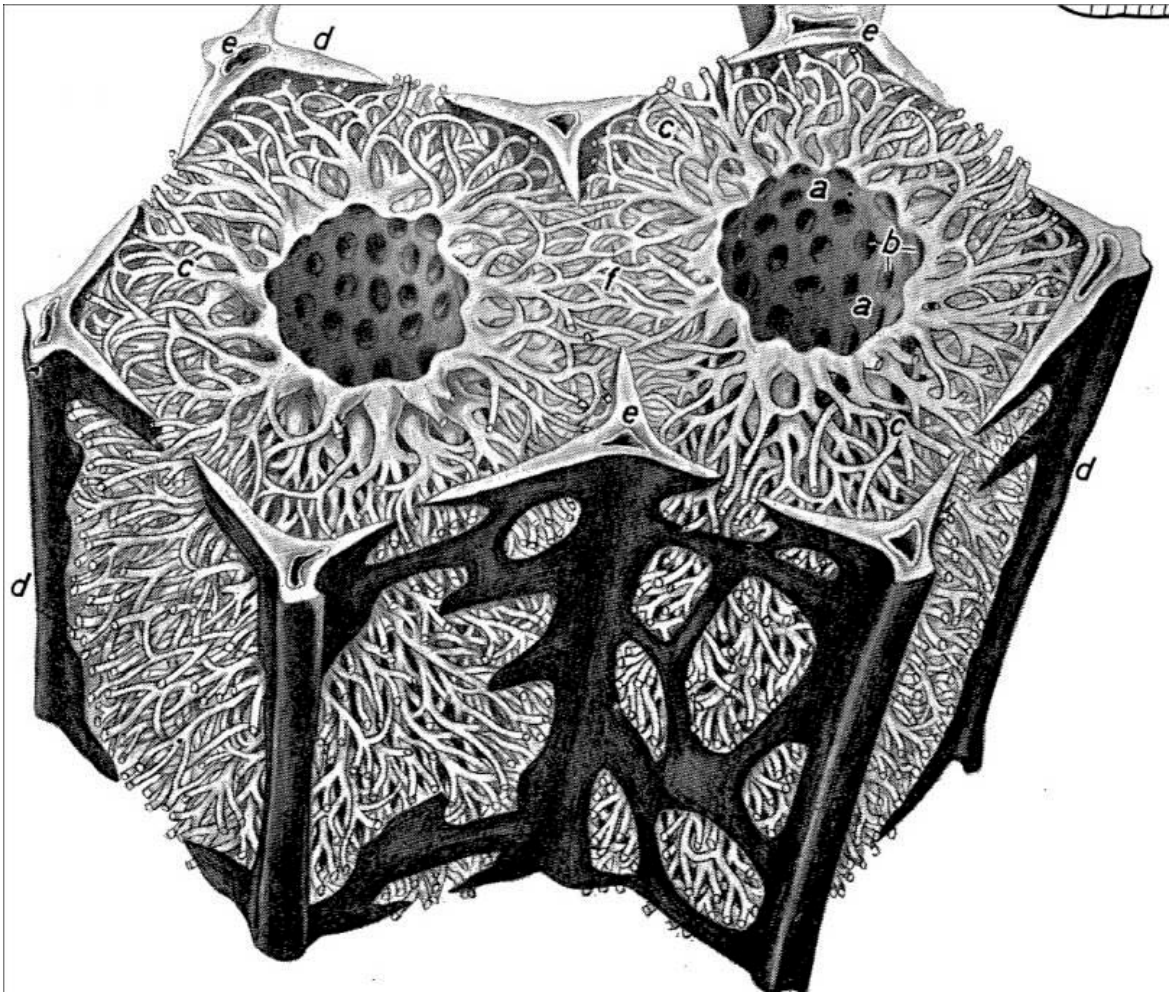


Figure 6. Section through two adjacent parabronchi. a: interatrial septa; b: atria; c: air capillaries; d: outer connective tissue septa; e: blood vessels; f: anastomotic connections between air capillaries. The **air capillaries** radiate outward toward the periphery (outer boundary) of the parabronchi.

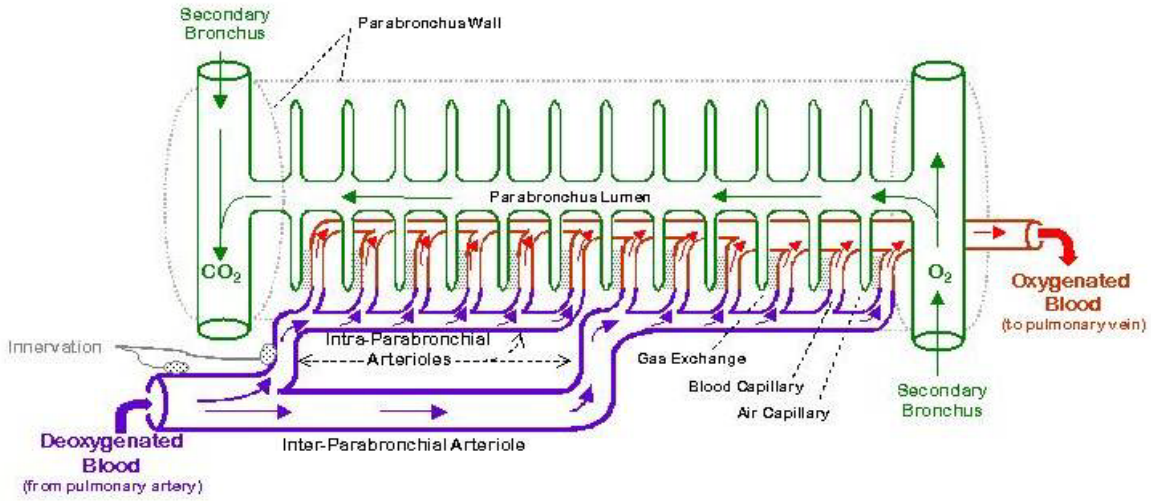


Figure 7. Interparabronchial arteries supply deoxygenated blood to Intraparabronchial arterioles branching inward into the parabronchial wall to form a net of blood capillaries surrounding each air capillary. Gas exchange occurs at the blood-gas barrier at the interface between blood capillaries and air capillaries. Venules collect the oxygenated blood at the base of the atria and infundibula adjacent to the parabronchial lumen.

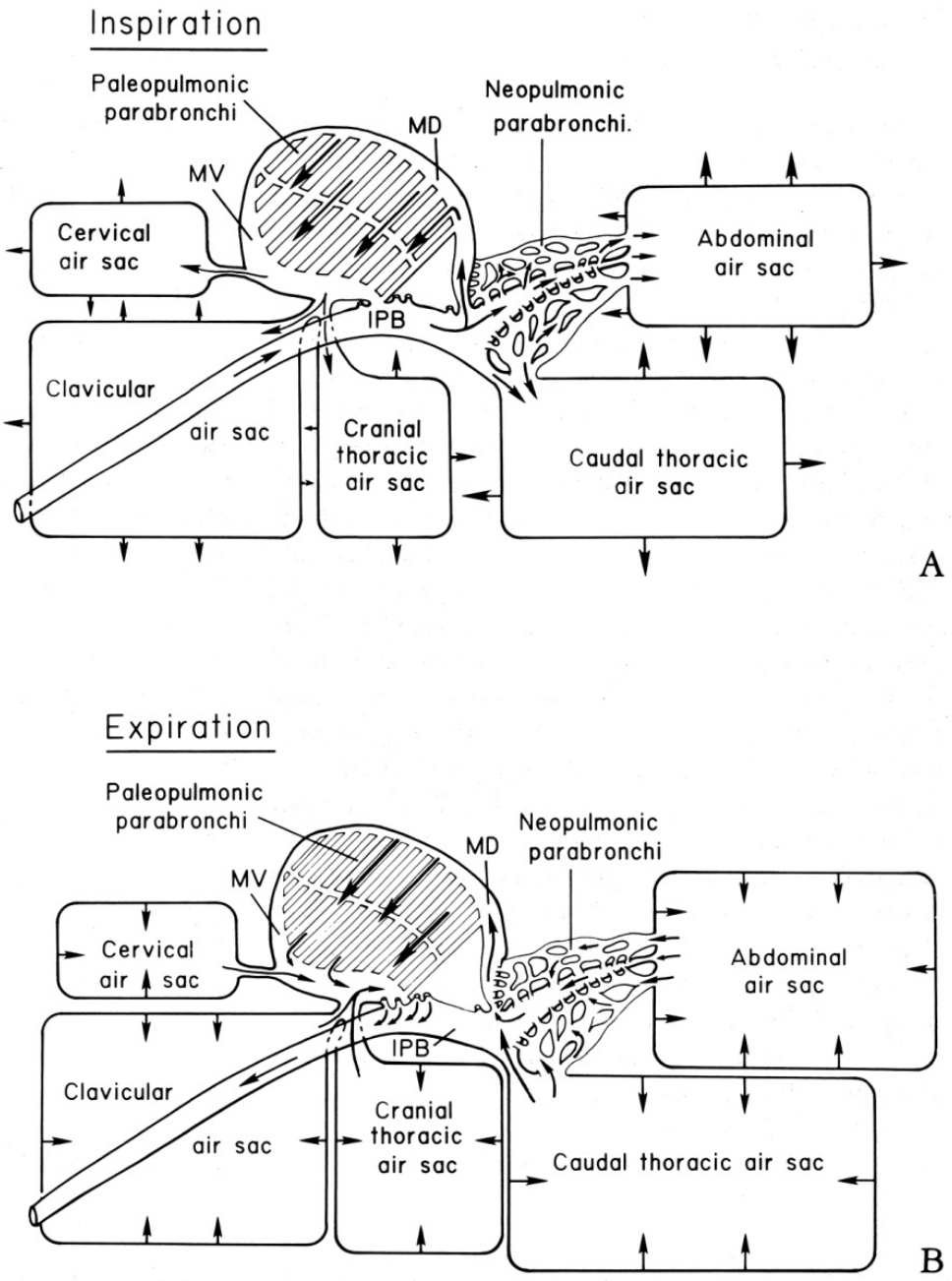


Figure 8. Schematic representation of the pathway of gas flow through the paleopulmonic and neopulmonic tertiary parabronchi during inspiration (A, upper panel) and expiration (B, lower panel). IPB: intrapulmonary primary bronchus; MD: mediodorsal secondary bronchi; MV: medioventral secondary bronchi. Outward arrows on air sacs (upper panel) = inflation caused by negative thoraco-abdominal pressures (suction); Inward arrows on air sacs (lower panel) = deflation caused by positive thoraco-abdominal pressures. Arrows in primary, secondary and tertiary parabronchi show directions of convective air flow.

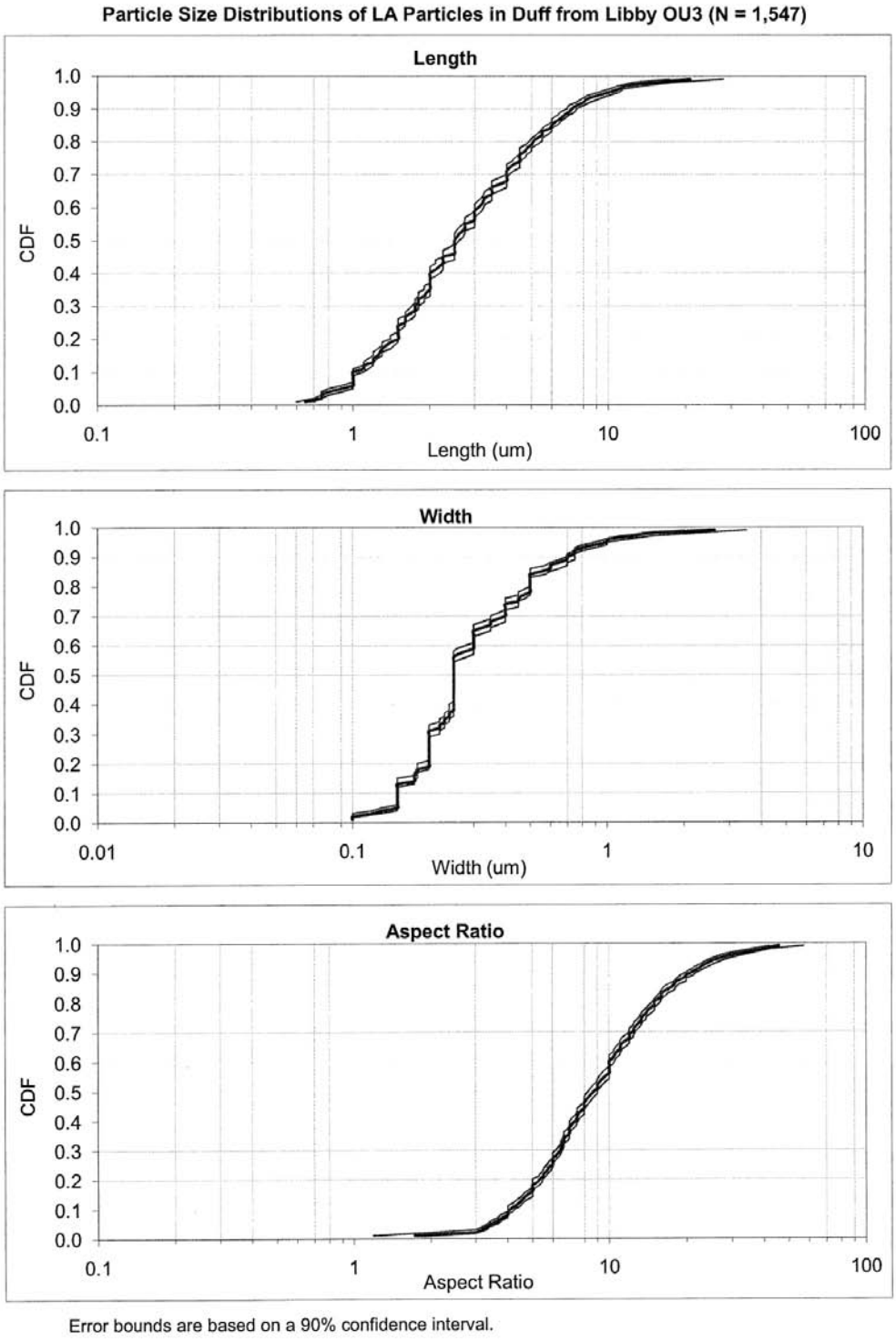


Figure 9. Particle size distributions for Libby Amphibole (LA) in duff.

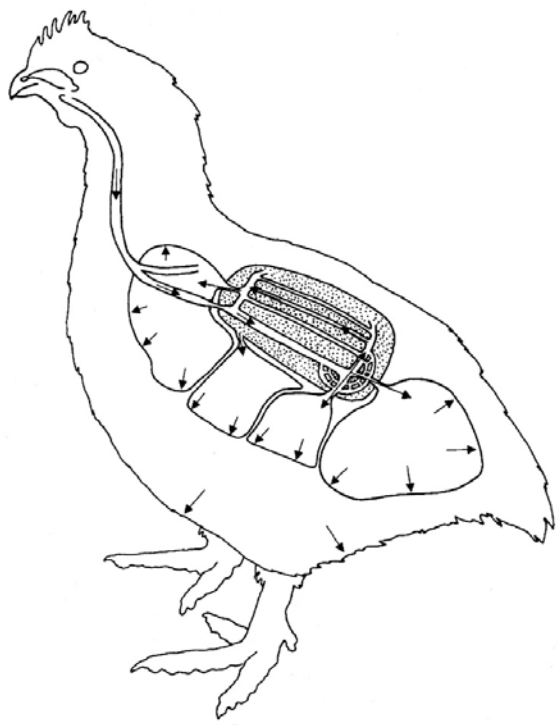


FIGURE 2. Pathway of gas flow in the avian respiratory system during inspiration. Enlargement of the body cavity by inspiratory muscle action lowers pressure in the air sacs relative to that in the atmosphere and gas flows into the system. Gas does not enter the medioventral secondary bronchi, but passes into the mediodorsal secondary bronchi. Some of the gas passes through the paleopulmonic parabronchi, and the remainder passes into the neopulmonic parabronchi and caudal air sacs.

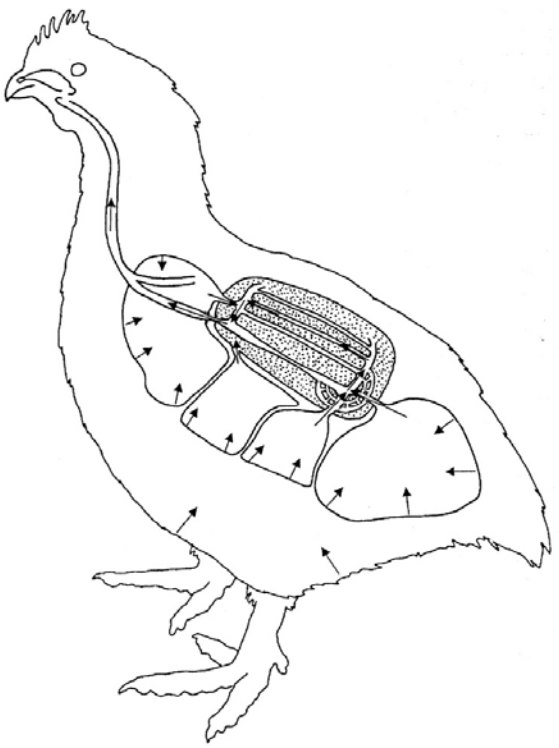


FIGURE 3. Pathway of gas flow in the avian respiratory system during expiration. Reduction in volume of the body cavity by expiratory muscle action increases pressure in the air sacs relative to that in the atmosphere and gas flows out of the system. Compression of intrapulmonary primary bronchus causes gas coming from the caudal air sacs to pass through neopulmonic parabronchi, into mediodorsal secondary bronchi and through the paleopulmonic parabronchi. Gas from the cranial air sacs does not pass through parabronchi on the way to the primary bronchus and trachea.

Figures from Fedde, 1998.